

# Heavier-than-air flying machines are impossible

L. Oliveira<sup>a</sup>, T. Hulsen<sup>b</sup>, D. Lutje Hulsik<sup>c</sup>, A.C.M. Paiva<sup>a</sup>, G. Vriend<sup>b,\*</sup>

<sup>a</sup>Escola Paulista de Medicina, Sao Paulo, Brazil

<sup>b</sup>CMBI, KUN, Toernooiveld 1, 6525 ED Nijmegen, The Netherlands

<sup>c</sup>Molecular Cell Biology, Utrecht University, Utrecht, The Netherlands

Received 17 December 2003; accepted 23 February 2004

First published online 26 March 2004

Edited by Fritz Winkler and Andreas Engel

**Abstract** Many G protein-coupled receptor (GPCR) models have been built over the years. The release of the structure of bovine rhodopsin in August 2000 enabled us to analyze models built before that period to learn more about the models we build today. We conclude that the GPCR modelling field is riddled with ‘common knowledge’ similar to Lord Kelvin’s remark in 1895 that “heavier-than-air flying machines are impossible”, and we summarize what we think are the (im)possibilities of modelling GPCRs using the coordinates of bovine rhodopsin as a template. Associated WWW pages: [http://www.gpcr.org/articles/2003\\_mod/](http://www.gpcr.org/articles/2003_mod/).

© 2004 Federation of European Biochemical Societies. Published by Elsevier B.V. All rights reserved.

**Key words:** GPCR; Modelling; Bovine rhodopsin; Bacteriorhodopsin; Superposition; Alignment

## 1. Introduction

G protein-coupled receptors (GPCRs) are the most important target for the pharmaceutical industry, as is indicated by the fact that 52% of all medicines available today act on them [1]. About 5500 GPCR sequences are publicly available. The GPCRDB [2] gives access to more than 10 000 mutations [3]. Binding constants are available for approximately 30 000 ligand–receptor combinations [2]. This wealth of sequences, ligands, and mutations contrasts sharply with the small amount of structural information.

Nearly all medicines are discovered by trial and error. Nevertheless, most pharmaceutical companies have large research departments that use every thinkable technique to design drugs. Homology modelling, as a tool to obtain structural information, is one of those techniques. In the past, bacteriorhodopsin [4–7] was often used as a modelling template, but recently the three-dimensional coordinates [8] of bovine rhodopsin have become available. It is a much better template for GPCR homology modelling than bacteriorhodopsin. However, bovine rhodopsin is not yet the perfect template, as will become clear later on in this article. Models produced Before the Crystal structure became available are called BC models, and those produced After these Data became available are called AD models.

### 1.1. BC modelling

Most BC models were based on either bacteriorhodopsin [4–7] or the C $\alpha$  coordinates of bovine rhodopsin derived by J. Baldwin [9] from an electron diffraction map [10–13]. A few models [14–16] were based on first principles, sometimes guided by a low-resolution electron diffraction map [10,13] of bovine or frog rhodopsin.

The BC modelling community developed a series of dogmas. E.g., a helix could not continue beyond the membrane region; loops were not supposed to dock between the helices; few models had helix kinks incorporated;  $\pi$ -helices and other irregularities were never considered; GPCRs had seven helices spanning the membrane; short loops had either no regular structure or the same structure as the isolated peptide in solution; the lysine in helix VII that binds the retinal is the same in bacteriorhodopsin and bovine rhodopsin. Generally the dogmatic BC model recipe was:

1. Determine which template to use, or design your own helix-packing model;
2. Use threading or moment calculations to determine the mapping of the GPCR sequence onto the selected template. Moment calculations can be based on hydrophobic moments [17], conservation moments [18], etc., or a combination of these [19]. Threading can be based on general rules, helix bundle rules [19,20], or even bacteriorhodopsin-specific rules [21];
3. Find experimental data that agree with the model and add them to convince yourself or the referees that this is the only correct model.

We found very many publications that discussed poor BC models, showing that things that are lighter than air will fly with referees and editors.

### 1.2. The bovine rhodopsin structure

The high-resolution structure of rhodopsin [8] reveals a seven-helix bundle with a central cavity surrounded by helices I–III and V–VII (see Fig. 1). Helix IV is not part of the cavity wall and makes contacts only with helix III. The central cavity is accessible from the cytosol, but the hairpin between helices IV and V prevents access from the periplasm. This hairpin lies between the helices, roughly parallel to the membrane surface. It has contacts with side chains of most of the helices. The most prominent contact is a disulfide bridge to helix III.

## 2. Methods

Data too numerous and hypotheses too speculative to be

\*Corresponding author. Fax: (31)-24-3652977.  
E-mail address: [vriend@cmbi.kun.nl](mailto:vriend@cmbi.kun.nl) (G. Vriend).

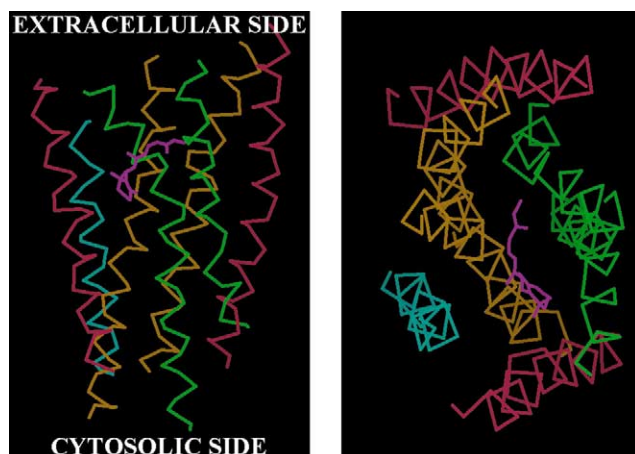


Fig. 1. The helix bundle in bovine rhodopsin. C $\alpha$  trace of bovine rhodopsin [8]. Retinal is shown in purple, helices II–III orange, VI–VII green, IV light blue, I and V red. Left: Side view. Right: Top view.

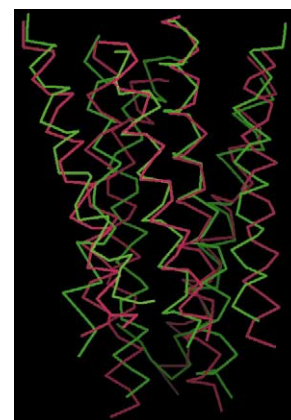


Fig. 2. Superposed bovine rhodopsin structure and model. The bovine rhodopsin structure [8] in red superposed in the GPCRDB [2] BC model built with WHAT IF [42], based on the C $\alpha$  coordinates provided by J. Baldwin [9].

put into print can be found at [http://www.gpcr.org/articles/2003\\_mod/](http://www.gpcr.org/articles/2003_mod/) [22]. This website also contains a detailed recipe for building models.

Bovine rhodopsin (PDBid = 1F88 [8]) and bacteriorhodopsin [4–6] are sufficiently differently organized to make any detailed structural comparison meaningless [10–12]. However, in order to evaluate the quality of models based on the bacteriorhodopsin template, this superposition must be made. We therefore did this structure superposition by hand. The recipe for determining the quality of bacteriorhodopsin-based BC models is as follows:

1. Extract from the GPCRDB the alignment of the sequence of the GPCR model with the sequence of bovine rhodopsin;
2. Use the superposed structures to align the bovine rhodopsin sequence onto the bacteriorhodopsin sequence;
3. Extract from the modelling article how the authors aligned their GPCR with bacteriorhodopsin. (If this alignment is not given, it can be extracted from a superposition of the bacteriorhodopsin-based GPCR model on the real bacteriorhodopsin structure.) This produces the alignment used for the modelling.

A comparison of the ‘optimal’ alignment with the alignment

		130			224
Rhodopsin	PWQFSMLAAYMFLIMLGF	INFLTYVTQ--		PLNYILLNLAVADLF	PMVFGGFTTLYTSLH
Bacteriorhodopsin	-----WNLALGTALMGL	LTLYPLVK----		-----PDAKKFYAIT	TLVPAIAPTMYLSMLL----
Cronet	-----VMVVGMI	VMSLIVLAIV	IGNVLI-- +4	-----LACADLV	GLAVVPFGAAILMKMW----
Vriend	-----QFSMLAAYMFLIMLGF	INFLTY-- +3		-----ILLNLAVADLF	PMVFGGFTTLYTSL-- +4
Kuipers	-----WVVGMAILMSVIVLAIV	IGNVLI-- +2		-----FITS	LACADLVMSGLAVVPFGASHIL-- +3
Rippmann	-----VTVSQVITSLLLGLTIFCAV	LGNACVAAIA +3		-----LERSLQNVANYLIGS	LAVTDLMSVSLVLPMAALYQVLN-- +4
		340			
Rhodopsin	GPTGCNLEGGFFATLGGEIAL	WSLVLAIERVVV-----			
Bacteriorhodopsin	-----IYWARYADWLF	FTPLLLDL-----			
Cronet	-----FWCEFWTSIDVLCVTAS	IELTLCVIAVD-- +11			
Vriend	-----GCNLEGGFFATLGGEIAL	WSLVLAIER-- +10			
Kuipers	-----CEFWTSIDVLCVTAS	IELTLCVIAVD-- +14			
Rippmann	-----KWTLGQVTCDLFIALD	VLCCTSSILHLCAIALDRY +17			
		420			520
Rhodopsin	ENHAIMGVAF	TVVMALACAAPPLV--		-----NESFVIYMFVVFHII	PLIVIFFCYQG-----
Bacteriorhodopsin	---QGTILALVG	ADGIMIGTGLVGAL		-----YSYRFVWMAIST	AAMLYILYVLF-----
Cronet	---TKNKARVILM	WIVSGLTSFL +4		-----NQ	YAIASSIYSPFYVPLVMV----- +4
Vriend	---HAIMGVAF	TVVMALACAAPPLV +1		-----FVIYMFVVFHII	PLIVIFFCY-- +6
Kuipers	---KARVILM	WIVSGLTSFLPIQ +1		-----YAIASSIYSPFYV	PLVMVVFY-- +6
Rippmann	-----SLTWLIGFLISIPPI--	-2		TPEDRSDPD	ACTISKDHGYTIYSTFGAFYIPLLLMLVLGRIFRAAR +5
		620			730
Rhodopsin	AEKEVTRMVIIMVIA	FLICWLFPYAGVAFYIFT----		-----PIFMTIPAFFAKTS	SAVYNPVIYIMMNKQFRNCMVITL
Bacteriorhodopsin	---PEVASTFKVL	RNVTVVLWSAYPVVWLI-----		-----ETLLFMVLDVS	AKVGFGLILLRSRA-----
Cronet	---TLGIIMGT	FLICWLFPFIVNIVHVIQ-- -4		-----EVYILLN	WIGYVNSQFNPLIYCRS----- +3
Vriend	---EVTMVIIMVIA	FLICWLFPYAGVAFY----- 0		-----DFGPIFMTIP	FAFFAKTSAVYNPVI----- +7
Kuipers	KALKTLGIIMGT	FLICWLFPFIVNIV-- -3		LIPKEVYILLN	WIGYVNSAFNPLI----- +2
Rippmann	-----TLGIIMGT	FLICWLFPFIVNIVLVPFCSSC 0		-----HMPTLLG	AINWLGYSNLSLNPVIYAYFNKD-- +7

Fig. 3. Sequence alignment extracted from deposited GPCR models produced by Cronet et al. [21], Oliveira et al. [44], Kuipers et al. [45,46], and Rippmann and Bottcher [47]. The top two lines show the alignment of bovine rhodopsin with bacteriorhodopsin. The motifs containing the most conserved residues in the GPCR transmembrane helices and the corresponding bacteriorhodopsin sequences are in red. The corresponding residues in the four models are light green. The vertical bars indicate the most conserved residue in each helix. The numbers behind these bars correspond to the GPCRDB [2] numbering schemes (the Cys in hairpin IV–V has number 480 in this numbering scheme). The numbers behind the sequences indicate the shift away from the perfect alignment. Minus signs indicate residues not available in the models. The fact that we could publish models that had residues misaligned by as many as 10 positions holds a warning for the future. It is important to realize that these alignments were (among) the best we could find in the literature that did not use the electron density-based C $\alpha$  coordinates extracted from Schertler’s electron density map by Baldwin.

used by the modeller is a good indication of the model quality. (This same method is used by the CASP competition judges to evaluate threading results [23].) Our recipe for obtaining these BC model alignment shifts differs, however, from what is normally used because only the structure of bovine rhodopsin is known, whilst the  $\beta_3$ -adrenergic receptor is the most-modelled GPCR.

### 3. Results and discussion

#### 3.1. The quality of BC models

Fig. 2 shows the superposition of the structure [8] and a very good BC model built, published [9], and deposited before August 2000. It can be seen that the gross features are modelled reasonably well. The C $\alpha$  and all-atom modelling errors (i.e. displacements between the model and the X-ray structure) are 2.5 Å and 3.2 Å, respectively. Although impressive, this model is still too bad to be of any use for ‘rational drug design’ purposes.

Bacteriorhodopsin and bovine rhodopsin are so different that quantitative structure comparisons are meaningless. We selected a superposition with a large overlap of the two retinal molecules. A shift in the structure superposition leads to a shift of three or four positions in the sequence alignment, which alternates between positive and negative in the seven helices. As can be seen from the alignments in Fig. 3, such a shift does not improve the alignments. Therefore, the subjective nature of the superposition does not influence our conclusions. We believe that all GPCR models (including our own) that are based on the bacteriorhodopsin template are bad, and none can have made a positive contribution to rational drug design projects. A more extensive discussion of BC models can be found in the article section of the GPCRDB [2].

No BC model had the IV–V hairpin located correctly between the helices. All modellers ‘knew’ that loop IV–V was external, but they were also aware of the disulfide bridge between helix III and this IV–V hairpin. Often bizarre reasoning was used to reconcile these two contradicting ‘facts’ and to justify the position of helix III. The experimental data enabling the correct prediction of the IV–V hairpin location were available to the BC modellers, because it was known that in opsins His474 and Lys477 in this hairpin form a chloride binding site that regulates the optimal absorption wavelength of the retinal [24]. It could have been reasoned that if this site modifies the wavelength, it should be located near the retinal. Unfortunately, the common ‘knowledge’ that the loops stick out into the solvent overcame the experimental data about the chloride site.

#### 3.2. The quality of AD models

We were surprised to find many modelling studies performed after the release of the bovine rhodopsin three-dimensional coordinates into which very little knowledge of this template was incorporated. Ballesteros et al. [25] recently wrote that amine receptors can be modelled from the bovine rhodopsin template. They neglect the IV–V hairpin, crystal contacts, and the fact that many residues cannot be detected in the X-ray structure. Orry et al. [26] docked endothelin in an endothelin receptor model based on a rhodopsin model by Pogozheva et al. [20]. They write in a note added after submission that the bovine rhodopsin structure became available

after the paper was first submitted, and claim that their model and the bovine rhodopsin structure are similar. Their model is not deposited, but from the figures in the article, it can be seen that the endothelin molecule is docked where one would expect the IV–V hairpin, and this hairpin is modelled as a hyper-exposed loop. These are just two of the many examples of neglect of details of the bovine rhodopsin structure. A survey of recent, GPCR modelling-related literature revealed a series of flaws:

1. Total neglect of loops and the IV–V hairpin [27–30];
2. Modelling loops based on data for individual loops obtained from nuclear magnetic resonance experiments or from sequence similarity with another PDB file [31–34];
3. Models in which molecular dynamics compacted the IV–V hairpin [35];
4. Models based on a frog C $\alpha$  map [36].

It is regrettable that a molecular dynamics publication on a homology model can be accepted for publication when the author has failed to show what the same protocol does to the bovine rhodopsin structure. All GPCR models are wrong, but some GPCR models can be useful [37]. Only the first part of this modification of a famous quote of G.E.P. Box, however, applies to the majority of the recently published GPCR models.

#### 3.3. AD GPCR modelling

The availability of the bovine rhodopsin structure opens new alleys for modelling GPCRs. However, some warnings are needed. First, the observed structure of many loops seems to be determined by crystal contacts. Second, the bovine rhodopsin structure is the inactive form of the protein, whilst the active form is a much more appropriate modelling goal for pharmaceutical purposes. Third, it is far from certain that the bovine rhodopsin structure can be used as a template for all GPCRs, because many sequence analyses indicate that opsins differ very much from the pharmaceutically interesting (class A) GPCRs. Fourth, the rhodopsin structure is an anti-parallel dimer, whereas GPCR dimers must be parallel.

Modelling studies start with a sequence alignment between the bovine rhodopsin template and the GPCR model sequence. The percentage sequence identity between bovine rhodopsin and many other (class A) GPCRs can be as low as 20%. Normally, when the sequence identity between the model and the template falls below 30%, the sequence alignment is the main bottleneck in the homology modelling procedure. Class A GPCRs are an exception to this rule, because each helix contains one or two highly conserved residues that allow an unambiguous alignment.

It is difficult to model the loops by homology, because most cytosolic loops cannot be seen in an electron density map, and most observed extracellular loop structures are probably induced by crystal packing forces. In any case, the sequence identity between most GPCRs and bovine rhodopsin is too low to derive any reliable loop alignment. At three locations, however, features can be seen that give hope for modelling. These are the highly conserved (details are provided in the WWW pages; numbering as in Fig. 3):

1. Trp280 and Gly295 in loop II–III;
2. Loop IV–V and the Cys315–Cys480 disulfide bridge;
3. Tyr734 at the bend between the helices VII and VIII and the adjacent sequence motif Phe800, Arg/Lys801 in helix VIII.



### 3.4. The active form

Modelling the active form of AGPCRs depends critically on the hypothesized mechanism of that activation process. We therefore start with a summary of possible activation mechanisms. These activation models consist of essentially the same three general steps:

1. Entry of the ligand into the ligand binding pocket;
2. The receptor moving from the inactive state into the active state, or the active state being frozen by the ligand;
3. The G protein being activated, or the activated state being frozen.

The clearest lesson to be learned from the BC experience is that molecular dynamics technology has not reached the level of maturity needed to aid in the prediction of the differences between the active and the inactive state.

### 3.5. New rules to replace the old dogmas

For most studies, it will be enough to model the seven transmembrane helices and the IV–V hairpin. If more loops are needed in the model, there is some hope for a few receptors that these loop models can be based on the structure, but in most cases it will not be possible to model them. The work by Yeagle et al. [38–41] makes clear that determination of the structure of the loops independently of the rest of the molecule is not successful.

The alignment of the helices should be based on the conserved motifs. Extrapolating from the performance of GPCR modellers over the years, we can only advise sticking to the bovine rhodopsin helix backbone coordinates. Any attempt to ‘improve’ this for other GPCRs will undoubtedly make things worse rather than better.

The IV–V hairpin should be modelled from bovine rhodopsin. If this loop is not present in the model sequence, it seems doubtful that a reliable model structure can be built.

If data exist that indicate dimer formation, these data must be used. Several studies, i.e. atomic force microscopy [43] work on mouse rhodopsins, can provide good information on how to model dimers, if needed.

The bovine rhodopsin three-dimensional coordinates represent the inactive form of this receptor. To model the (pharmacologically much more interesting) active form of GPCRs, one should not rely on molecular dynamics, but rather on the outcome of experiments that can be interpreted unambiguously.

Our WWW pages list a recipe for modelling the active form of a receptor. One day, this recipe will be proven wrong, but it is the best we can do given current data and Occam’s razor.

**Acknowledgements:** The GPCRDB is an EC-sponsored project (PL 950224). The authors thank Margot Beukers, Bob Bywater, Oyvind Edvardsen, Florence Horn, Rob Hooft, Wilma Kuipers, Friedrich Rippmann, Chris Sander, Mike Singer, Nora v.d. Wenden, and Ad IJzerman for stimulating discussions. We thank David Thomas for critically reading the manuscript.

## References

- [1] Watson, S. and Arkinstall, S. (1994) The G-protein Linked Receptor Facts Book, Academic Press, New York.
- [2] Horn, F., Weare, J., Beukers, M.W., Horsch, S., Bairoch, A., Chen, W., Edvardsen, O., Campagne, F. and Vriend, G. GPCRDB: an information system for G protein-coupled receptors, (1998) *Nucleic Acids Res.* 26, 275–279.
- [3] Beukers, M.W., Kristiansen, I., IJzerman, A.P. and Edvardsen, O. TinyGRAP database: a bioinformatics tool to mine G protein-coupled receptor mutant data, (1999) *Trends Pharmacol. Sci.* 20, 475–477.
- [4] Pebay-Peyroula, E., Rummel, G., Rosenbusch, J.P. and Landau, E.M. X-ray structure of bacteriorhodopsin at 2.5 angstroms from microcrystals grown in lipid cubic phases, (1997) *Science* 277, 1676–1681.
- [5] Luecke, H., Richter, H.T. and Lanyi, J.K. Proton transfer pathways in bacteriorhodopsin at 2.3 Ångstrom resolution, (1998) *Science* 280, 1934–1937.
- [6] Takeda, K., Sato, H., Hino, T., Kono, M., Fukuda, K., Sakurai, I., Okada, T. and Kouyama, T. A novel three-dimensional crystal of bacteriorhodopsin obtained by successive fusion of the vesicular assemblies, (1998) *J. Mol. Biol.* 283, 463–474.
- [7] Henderson, R. and Schertler, G.F.X. The structure of bacteriorhodopsin and its relevance to the visual opsins and other seven-helix G protein-coupled receptors, (1990) *Phil. Trans. R. Soc. Lond. B Biol. Sci.* 326, 379–389.
- [8] Palczewski, K., Kumasaka, T., Hori, T., Behnke, C.A., Motoshima, H., Fox, B.A., Le Trong, I., Teller, D.C., Okada, T., Stenkamp, R.E., Yamamoto, M. and Miyano, M. Crystal structure of rhodopsin: a G protein-coupled receptor, (2000) *Science* 289, 739–745.
- [9] Baldwin, J.M. The probable arrangement of the helices in G protein-coupled receptors, (1993) *EMBO J.* 12, 1693–1703.
- [10] Unger, V.M. and Schertler, G.F.X. Low resolution structure of bovine rhodopsin determined by electron cryo-microscopy, (1995) *Biophys. J.* 68, 1776–1786.
- [11] Schertler, G.F., Villa, C. and Henderson, R. Projection structure of rhodopsin, (1993) *Nature* 362, 770–772.
- [12] Unger, V.M., Hargrave, P.A., Baldwin, J.M. and Schertler, G.F.X. Arrangement of rhodopsin transmembrane alpha-helices, (1997) *Nature* 389, 203–206.
- [13] Schertler, G.F.X. and Hargrave, P.A. Projection structure of frog rhodopsin in two crystal forms, (1995) *Proc. Natl. Acad. Sci. USA* 92, 11578–11582.
- [14] Filizola, M., Perez, J.J. and Carteni-Farina, M. BUNDLE: A program for building the transmembrane domains of G protein-coupled receptors, (1998) *J. Comput.-Aid. Mol. Des.* 12, 111–118.
- [15] Prusis, P., Schiöth, H.B., Muceniece, R., Herzyk, P., Afshar, M., Hubbard, R.E. and Wikberg, J.E.S. Modelling of the three-dimensional structure of the human melanocortin 1 receptor, using an automated method and docking of a rigid cyclic melanocyte-stimulating hormone core peptide, (1997) *J. Mol. Graph. Mod.* 15, 307–315.
- [16] Bramblett, R.D., Panu, A.M., Ballesteros, J.A. and Reggio, P.H. Construction of a 3D model of the cannabinoid CB1 receptor: determination of helix ends and helix orientation, (1995) *Life Sci.* 56, 1971–1982.
- [17] Donnelly, D., Overington, J.P., Ruffle, S.V., Nugent, J.H. and Blundell, T.L. Modelling alpha-helical transmembrane domains: the calculation and use of substitution tables for lipid-facing residues, (1993) *Protein Sci.* 2, 55–70.
- [18] Pardo, L., Ballesteros, J.A., Osman, R. and Weinstein, H. On the use of the transmembrane domain of bacteriorhodopsin as a template for modeling the three-dimensional structure of guanine nucleotide-binding regulatory protein-coupled receptors, (1992) *Proc. Natl. Acad. Sci. USA* 89, 4009–4012.
- [19] Herzyk, P. and Hubbard, R.E. Combined biophysical and biochemical information confirms arrangement of transmembrane helices visible from the three-dimensional map of frog rhodopsin, (1998) *J. Mol. Biol.* 281, 741–754.
- [20] Pogozheva, I.D., Lomize, A.L. and Mosberg, H.I. The transmembrane 7-alpha-bundle of rhodopsin: distance geometry calculations with hydrogen bonding constraints, (1997) *Biophys. J.* 72, 1963–1985.
- [21] Cronet, P., Sander, C. and Vriend, G. Modelling of transmembrane seven helix bundles, (1993) *Protein Eng.* 6, 59–64.
- [22] [http://www.gpcr.org/articles/2003\\_mod/index.html](http://www.gpcr.org/articles/2003_mod/index.html).
- [23] Venclovas, C., Zemla, A., Fidelis, K. and Moult, J. Comparison of performance in successive CASP Experiments, (2001) *Proteins* 5 (Suppl.), 163–170.
- [24] Wang, Z., Asenjo, A.B. and Oprian, D.D. Identification of the

- Cl<sup>-</sup>-binding site in the human red and green colour vision pigments, (1993) *Biochemistry* 32, 2125–2130.
- [25] Ballesteros, J.A., Shi, L. and Javitch, J.A. Structural mimicry in G protein-coupled receptors: implications of the high-resolution structure of rhodopsin for structure-function analysis of rhodopsin-like receptors, (2001) *Mol. Pharmacol.* 60, 1–19.
- [26] Orry, A.J.W. and Wallace, B.A. Modelling and docking the endothelin G protein-coupled receptor, (2000) *Biophys. J.* 79, 3083–3094.
- [27] Lopez-Rodriguez, M.L., Murcia, M., Benhamu, B., Olivella, M., Campillo, M. and Pardo, L. Computational model of the complex between GR113808 and the 5-HT<sub>4</sub> receptor guided by site-directed mutagenesis and the crystal structure of rhodopsin, (2001) *J. Comput.-Aid. Mol. Des.* 15, 1025–1033.
- [28] Lopez-Rodriguez, M.L., Vicente, B., Deupi, X., Barrondo, S., Olivella, M., Morcillo, M.J., Behamu, B., Ballesteros, J.A., Salles, J. and Pardo, L. Design, synthesis and pharmacological evaluation of 5-hydroxytryptamine(1a) receptor ligands to explore the three-dimensional structure of the receptor, (2002) *Mol. Pharmacol.* 62, 15–21.
- [29] Shim, J.Y., Welsh, W.J. and Howlett, A.C. Homology model of the CB1 cannabinoid receptor: Sites critical for nonclassical cannabinoid agonist interaction, (2003) *Biopolymers* 71, 169–189.
- [30] Vaidehi, N., Floriano, W.B., Trabanino, R., Hall, S.E., Freddolino, P., Choi, E.J., Zamanakos, G. and Goddard III, W.A. Prediction of structure and function of G protein-coupled receptors, (2002) *Proc. Natl. Acad. Sci. USA* 99, 12622–12627.
- [31] Lequin, O., Bolbach, G., Frank, F., Convert, O., Girault-Lagrange, S., Chassaing, G., Lavielle, S. and Sagan, S. Involvement of the second extracellular loop (E2) of the neurokinin-1 receptor in the binding of substance P. Photoaffinity labeling and modeling studies, (2002) *J. Biol. Chem.* 277, 22386–22394.
- [32] Chung, D.A., Zuiderweg, E.R., Fowler, C.B., Soyer, O.S., Mosberg, H.I. and Neubig, R.R. NMR structure of the second intracellular loop of the alpha 2A adrenergic receptor: evidence for a novel cytoplasmic helix, (2002) *Biochemistry* 41, 3596–3604.
- [33] Yang, X., Wang, Z., Dong, W., Ling, L., Yang, H. and Chen, R. Modeling and docking of the three-dimensional structure of the human melanocortin 4 receptor, (2003) *J. Protein Chem.* 22, 335–344.
- [34] Mehler, E.L., Periole, X., Hassan, S.A. and Weinstein, H. Key issues in the computational simulation of GPCR function: representation of loop domains, (2002) *J. Comput.-Aid. Mol. Des.* 16, 841–853.
- [35] Pellegrini, M., Bremer, A.A., Ulfers, A.L., Boyd, N.D. and Mierke, D.F. Molecular characterization of the substance P<sup>\*</sup>neurokinin-1 receptor complex: development of an experimentally based model, (2001) *J. Biol. Chem.* 276, 22862–22867.
- [36] Church, W.B., Jones, K.A., Kuiper, D.A., Shine, J. and Iismaa, T.P. Molecular modelling and site-directed mutagenesis of human GALR1 galanin receptor defines determinants of receptor subtype specificity, (2002) *Protein Eng.* 5, 313–323.
- [37] Shi, L. and Javitch, J.A. The binding site of aminergic G protein-coupled receptors: the transmembrane segments and second extracellular loop, (2002) *Annu. Rev. Pharmacol. Toxicol.* 42, 437–467.
- [38] Yeagle, P.L., Alderfer, J.L. and Albert, A.D. Structure determination of the fourth cytoplasmic loop and carboxyl terminal domain of bovine rhodopsin, (1996) *Mol. Vis.* 2, 12–19.
- [39] Yeagle, P.L., Alderfer, J.L., Salloum, A.C., Ali, L. and Albert, A.D. The first and second cytoplasmic loops of the G protein-coupled receptor, rhodopsin, independently form beta-turns, (1997) *Biochemistry* 36, 3864–3869.
- [40] Yeagle, P.L., Alderfer, J.L. and Albert, A.D. Structure of the third cytoplasmic loop of bovine rhodopsin, (1995) *Biochemistry* 34, 14621–14625.
- [41] Yeagle, P.L., Salloum, A., Chopra, A., Bhawsar, N., Ali, L., Kuzmanovski, G., Alderfer, J.L. and Albert, A.D. Structures of the intradiskal loops and amino terminus of the G-protein receptor, rhodopsin, (2000) *J. Pept. Res.* 55, 455–465.
- [42] Vriend, G. WHAT IF: A molecular modeling and drug design program, (1990) *J. Mol. Graph.* 8, 52–56.
- [43] Liang, Y., Fotiadis, D., Filipek, S., Saperstein, D.A., Palczewski, K. and Engel, A. Organization of the G protein-coupled receptors rhodopsin and opsin in native membranes, (2003) *J. Biol. Chem.* 278, 21655–21662.
- [44] Oliveira, L., Paiva, A.C.M. and Vriend, G. A common motif in G protein-coupled seven transmembrane helix receptors, (1993) *J. Comput.-Aid. Mol. Des.* 7, 649–658.
- [45] Kuipers, W., Van Wijngaarden, I. and IJzerman, A.P. A model of the serotonin 5-HT<sub>1A</sub> receptor: agonist and antagonist binding sites, (1994) *Drug Des. Discov.* 11, 231–249.
- [46] Kuipers, W., Oliveira, L., Vriend, G. and IJzerman, A.P. Identification of class-determining residues in G protein-coupled receptors by sequence analysis, (1997) *Receptors Channels* 5, 159–174.
- [47] Rippmann, F. and Bottcher, E. Molecular modelling of serotonin receptors, (1993) *7TM* 3, 1–27.